Cellular/Molecular

Clearance of α-Synuclein Oligomeric Intermediates via the Lysosomal Degradation Pathway

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Cytoplasmic deposition of α-synuclein aggregates is a common pathological feature of many neurodegenerative diseases. Strong evidence for the causative role of α-synuclein in these disorders is provided by genetic linkage between this gene and familial Parkinson's disease and by neurodegeneration in transgenic animals that overexpress this protein. In particular, it has been hypothesized that the accumulation of nonfibrillar oligomers of α-synuclein, which serve as intermediates for fibrillar inclusion body formation, causes neurodegeneration. However, little is known about how cells handle potentially toxic protein aggregates. Here we demonstrate that cells are capable of clearing preformed α-synuclein aggregates via the lysosomal degradation pathway. Consequently, blocking this pathway causes the accumulation of the aggregates in non-neuronal cells, differentiated neuroblastoma cells, and primary cortical neurons. This aggregate clearance occurs in an aggregation stage-specific manner; oligomeric intermediates are susceptible to clearance, whereas mature fibrillar inclusion bodies are not. Neutralization of the acidic compartments leads to the accumulation of α-synuclein aggregates and exacerbates α-synuclein toxicity in postmitotic neuronal cells, suggesting that the accumulation of oligomeric intermediates may be an important event leading to α-synuclein-mediated cell death. These results suggest that enhancing lysosomal function may be a potential therapeutic strategy to halt or even prevent the pathogenesis of Parkinson's disease and other Lewy body diseases.

Key words: α-synuclein; protein aggregation; lysosome; Parkinson's disease; Lewy body; neurodegeneration

Introduction

Deposition of filamentous α-synuclein (α-syn) in the neuronal or glial cytoplasm is a common pathological feature of many neurological diseases, such as Parkinson's disease (PD), dementia with Lewy bodies, multiple system atrophy, and neuronal degeneration with brain iron accumulation type 1 (Trojanowski et al., 1998; Goedert, 2001). All of the genetic variations in human α-syn gene that are causative to the early-onset familial parkinsonism increase the probability to form aggregates (Polymeropoulos et al., 1997; Kruger et al., 1998; Conway et al., 2000; Singleton et al., 2003). Animal models developed in mice and flies have shown that overexpression of α-syn in neurons can cause neuronal loss, along with α-syn aggregation that leads to the formation of both filamentous and granular aggregates (Feany and Bender, 2000; Masliah et al., 2000; Giasson et al., 2002; M. Lee et al., 2002), further supporting the hypothesis that abnormal accumulation of α-syn aggregates may play a critical role in the pathogenesis of neurodegenerative diseases.

Biochemical studies have shown that α-syn can form amyloid-like fibrils with a cross-β-sheet conformation (Serpell et al., 2000). These fibrils have a similar morphology to that found in Lewy bodies (LBs) (Spillantini et al., 1998), suggesting that the mechanism of cell-free fibrillation may reflect the actual pathogenic process *in vivo*. Fibrillation of α-syn initiates with the dimerization of partially folded monomers (Uversky et al., 2001; Krishnan et al., 2003), followed by the formation of β-sheet-rich nonfibrillar oligomeric intermediates, also known as protofibrils, with several distinct morphologies (Volles and Lansbury, 2003). More recently, we have developed a cell culture model in which overexpression of human α-syn leads to the formation of LB-like inclusion bodies that consist mainly of fibrillar aggregates. In these cells we have demonstrated that nonfibrillar spherical oligomers act as intermediates in the formation of fibrillar inclusion bodies and that the oligomer-to-fibril transition requires microtubule-dependent deposition of oligomers in the pericentriolar region (Lee and Lee, 2002). The mechanism underlying the deleterious effects of α-syn aggregates remains to be elucidated. Several studies have suggested that the oligomeric intermediates are the cause of cellular dysfunction and cell death (Gosavi et al., 2002; Kayed et al., 2003; Volles and Lansbury, 2003), whereas others have pointed to the direct role of fibrillar inclusion bodies in neurodegeneration (for review, see Giasson and Lee, 2003).

The extent of aggregate accumulation likely is determined by a dynamic equilibrium between the production and clearance of aggregates, and this process may, in turn, be critical for cell viability. In contrast to the rapid progress in our understanding of the α-syn aggregation process and its regulation, little is known about the breakdown of preformed aggregates. Previously, we have shown that α-syn aggregation can be promoted by treating

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